The neurobiology of adolescent nicotine exposure

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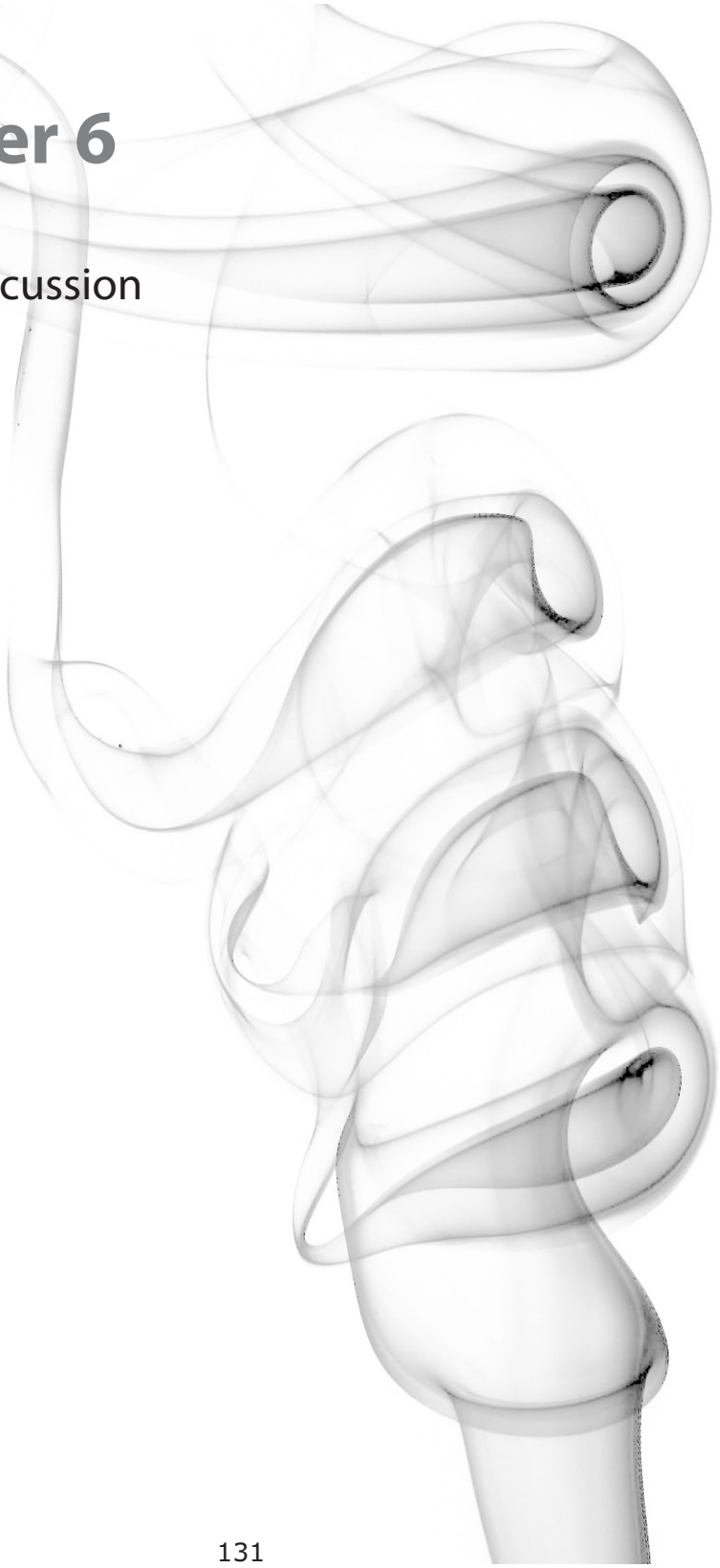
Chapter 6

General discussion



1. Summary

Adolescence is a period of increased vulnerability to the long-term effects of drugs of abuse, such as nicotine from tobacco smoking. Adolescent nicotine exposure in humans has been shown to cause both short-term decrements in attentional performance (Jacobsen *et al,* 2005), and epidemiological research suggests long-lasting effects that encompass an increased chance of developing behavioral problems and psychiatric disorders (Mathers *et al,* 2006). However, from these human data it is unclear whether these effects are directly caused by adolescent nicotine use, and the underlying molecular en cellular mechanisms are unknown. The first aim of my thesis was to investigate the molecular differences between adolescent and adult synapses in the rat mPFC (Chapter 2). My second aim was to investigate the long-term effects of adolescent nicotine exposure on cognitive performance (Chapter 3). In Chapter 4 and 5, I addressed the third aim, which was to find the molecular and physiological mechanisms that mediate these long-lasting effects of adolescent nicotine exposure on attention and impulsivity. In Chapter 4, I describe the expression of nicotinic receptors in the mPFC both one day and five weeks following adolescent nicotine exposure. Finally, in Chapter 5 I used a proteomics approach to find synaptic proteins that are differentially expressed due to adolescent nicotine exposure to investigate the link between synaptic proteins in the mPFC that are affected by adolescent nicotine exposure on the long-term and behavior in a cognitive paradigm.

1.1 Scope of the general discussion

I will now review these topics in the light of recent work, and give an overview of the long-term effects of adolescent exposure to drugs, including my own findings that adolescent nicotine exposure leads to long-term decrements in cognitive performance. Next, I will examine why the adolescent brain is more vulnerable to the effects of drugs of abuse, and discuss my findings on the adolescent development of synapses in the mPFC. Finally, I will review the functional consequences of adolescent nicotine exposure, both on the short-term, where we observed differences in the expression of nicotinic receptors and subtle differences in the expression of glutamate receptors at the synapse, and on the long-term, where I revealed some of the synaptic mechanisms that are associated with behavioral changes that follow adolescent nicotine exposure.

2. Drug abuse and the adolescent brain

Adolescence represents a period of gradual transition from childhood to

adulthood, and is a developmental phase that is conserved between mammalian species (for review (Spear, 2000)). Adolescence is characterized by an increase in peer-directed interactions, risk-taking and impulsive behavior, and is therefore a period in which individuals will become independent from their parents and move away from their home environment. From an evolutionary perspective, the conservation of this period is thought to be useful in order to protect a group of animals or humans from inbreeding, or to help them to get acquainted with new types of food that the more impulsive adolescents will have tried first.

Increased risk-taking seems to result from an increase in novelty and sensation seeking, and a not yet fully developed system of self-regulatory competence (Steinberg, 2004). When adolescents are compared with either adults or children, it appears that neural activity is different in response to reward (Galvan *et al,* 2006; Van Leijenhorst *et al,* 2009). This is exemplified by the exaggerated activity in the nucleus accumbens relative to the prefrontal cortex that was observed in adolescents using functional magnetic resonance imaging (fMRI), when exposed to reward value manipulations (Galvan *et al,* 2006). An alternative explanation for increased risk taking is a decreased sensitivity of the ventral striatum to reward in adolescents compared with adults (Bjork *et al,* 2004), which has been suggested to cause adolescents to seek more stimulating experiences. Both the fact that adolescents are more likely to take risks and act impulsive, and the fact that they may perceive drugs as more rewarding might cause many adolescents to experiment with drugs.

2.1 Consequences of drug exposure to the adolescent brain

Drugs of abuse are known to cause enduring changes in the (adult) brain, and specifically in the mesocorticolimbic system (for review, i.e. (Dietz *et al,* 2009; Kauer and Malenka, 2007; Thomas *et al,* 2008)). These changes are thought to underlie the addictive properties of these substances. Given the fact that the adolescent brain is still developing, drugs administered to adolescents might have more dramatic effects than in adults, leading to long-lasting molecular, synaptic and behavioral changes. For example, individuals that begin drug use early in adolescence show a pattern of higher lifetime consumption and more difficulty quitting (Breslau and Peterson, 1996; Chen and Millar, 1998; Mackesy-Amiti *et al,* 1997). This exaggerated effect of drugs of abuse during adolescence is of great interest regarding the fact that the majority of adult drug users have started experimenting with drugs during adolescence (Chambers *et al,* 2003). Not only is early drug use a predictor for addictive behavior in later

life, also epidemiological research suggests that early drug exposure can lead to an increased risk for cognitive and emotional disorders in adulthood, which has been documented for nicotine, alcohol and cannabis use (Brown *et al,* 2008; Jacobus *et al,* 2009; Mathers *et al,* 2006). However, in humans it is difficult to assess whether early drug exposure affects brain development in a way that leads to a pathological outcome, or whether genetic and/or environmental factors underlie both early drug taking and subsequent psychopathology; thus leaving the issue of 'cause and consequence' unresolved.

Animal models have greatly contributed to our understanding of the long-term effects caused by adolescent drug exposure. There are many examples of studies in which the long-term effects of adolescent exposure to alcohol, nicotine, cannabinoids and stimulants have been assessed. Adolescence appears to be a period of increased vulnerability to the development of long-term effects following drug exposure. Regardless of the type of drug of abuse administered, adolescent drug exposure causes an increase in reward-related behavior in most (Adriani *et al,* 2006b; Adriani *et al,* 2003; Biscaia *et al,* 2008; Brenhouse *et al,* 2009; Diaz-Granados and Graham, 2007; Fone *et al,* 2002; Hutchison and Riley, 2008; Kota *et al,* 2009; Santos *et al,* 2009), but not all (Kelley and Rowan, 2004; Schneider and Koch, 2003) cases and disrupts cognitive or executive functioning (Adriani *et al,* 2007; Adriani *et al,* 2006b; Black *et al,* 2006; Li *et al,* 2009; Nasrallah *et al,* 2009; Schneider *et al,* 2003; Wegener and Koch, 2009). Whether the underlying mechanisms behind this alteration of addictive behavior and cognitive performance are interrelated had never been tested, but this seems likely, e.g. regarding the relationship between impulsivity and drug addiction (Diergaarde *et al,* 2008).

2.2 Nicotine exposure during adolescence has long-lasting cognitive effects

I have added new data to this concept by showing that adolescent, but not adult nicotine exposure has long-term effects on cognitive functioning, in that it decreases visuospatial attention and increases impulsive action, but not impulsive choice (Chapter 3). Regarding the effects of drug exposure on anxiety and exploration, apparently conflicting results have been reported. In some cases drug exposure increased anxiety and depression-like phenotypes (Abreu-Villaca *et al,* 2008; Faria *et al,* 2006; Iniguez *et al,* 2009; Rubino *et al,* 2008; Smith *et al,* 2006), but others have reported that drug exposure decreases these parameters (Adriani

et al, 2004a; Biscaia *et al,* 2003; Daza-Losada *et al,* 2008; O'Shea *et al,* 2006). Concerning long-term effects on paradigms measuring different aspects of learning and memory, adolescent drug exposure causes even more divergent results (Abreu-Villaca *et al,* 2007; Bergstrom *et al,* 2006; Bethancourt *et al,* 2009; Featherby *et al,* 2008; O'Shea *et al,* 2006; Pascual *et al,* 2007; Santucci, 2008; Schneider *et al,* 2003; Schneider *et al,* 2008; Smith *et al,* 2006; Vorhees *et al,* 2005), perhaps because the various employed learning paradigms (i.e. spatial learning, fear learning and object recognition) recruit different molecular and anatomical substrates. However, taken together all behavioral studies, adolescent drug exposure has a more dramatic and lasting effect than adult drug exposure.

Adolescent drug exposure has also been shown to lead to various long-term changes on the cellular (Brandon *et al,* 2003; Criado *et al,* 2008; Pistis *et al,* 2004; Slawecki, 2002; Slawecki *et al,* 2001; Slawecki and Ehlers, 2002) and molecular (Adriani *et al,* 2004a; Adriani *et al,* 2006b; Adriani *et al,* 2003; Camarini *et al,* 2008; Daza-Losada *et al,* 2008; Diaz Heijtz *et al,* 2003; Falco *et al,* 2009; Faria *et al,* 2006; Featherby *et al,* 2008; Marco *et al,* 2007; Ribeiro-Carvalho *et al,* 2009; Rubino *et al,* 2008; Slawecki *et al,* 2005a; Slotkin and Seidler, 2009; Soderstrom *et al,* 2007; Trauth *et al,* 2001; Trauth *et al,* 2000a; Wegener *et al,* 2009) level, for example long-lasting changes in gene expression (Adriani *et al,* 2006b; Adriani *et al,* 2003; Falco *et al,* 2009; Featherby *et al,* 2008; Soderstrom *et al,* 2007) and protein expression (Adriani *et al,* 2004a; Marco *et al,* 2007; Rubino *et al,* 2008). However, none of these changes have been shown to be directly responsible for any of the observed behavioral alterations following adolescent drug exposure. In my studies, I aimed to investigate which molecular changes and mechanisms are causative to the decrements in visuospatial attention and increments in impulsive action measured in the 5-CSRTT five weeks following adolescent nicotine exposure. Below, I will first discuss why the adolescent brain is particularly vulnerable to long-term changes brought about by adolescent drug exposure. Then I will present a model explaining how the short-term changes can lead to long-term changes in the mPFC.

3. What is so special about the adolescent brain?

Observing that adolescent animals are more prone to persistent effects of drug exposure, leads to the question why they are more vulnerable. This is an important question to answer, since most people start experimenting with drugs of abuse during adolescence. One answer to why adolescents are more vulnerable to these long-term changes would be that the

adolescent brain is more plastic than the adult brain, because it is still developing. The most important ongoing processes are synaptic pruning of excessive synapses, innervation from other brain regions, myelination of long-range connections and adaptation of neurotransmitter levels, causing differences in synapse function (reviewed in Chapter 1).

For the purpose of this thesis, I will focus on the mPFC, a cortical association area developing relatively late in time. This brain region keeps environmental representations that are held "on-line" in the absence of input, and inhibits inappropriate responses and environmental distraction (Arnsten *et al,* 2005). Also, descending projections from the mPFC can modulate the activity of neurons in the nucleus accumbens (Carr *et al,* 1999), which mediate the reinforcing effects of drugs of abuse and subsequent drug-seeking behavior (Di Chiara, 2002).

3.1 Maturation of synapses

Drugs of abuse are able to modify synaptic plasticity in the mesocorticolimbic system (Kauer *et al,* 2007). I will next examine whether adolescent synapses are indeed more plastic to begin with, and whether I found evidence of synapse elimination and/or maturation in the adolescent rat brain.

Although the majority of electrophysiological studies are performed with brain slices from juvenile animals, increasing evidence suggests that there are differences in the physiological properties of synapses in adolescents compared with adults. It seems that although most cortical synapses have been formed before P35, morphological and physiological parameters are still maturing during adolescence (Tseng, 2007). In the nucleus accumbens, it was shown that synapses onto medium spiny neurons continue to develop during adolescence, since in adults (P120-P200) the intrinsic excitability is reduced compared with both adolescent (P32-P42) and juvenile (P14-P21) animals (Kasanetz and Manzoni, 2009). Also, during adolescence the AMPAR EPSCs become slower and the heterogeneity of AMPAR/NMDAR ratios increases, suggesting an ongoing synaptic refinement (Kasanetz *et al,* 2009). Moreover, it is more difficult to induce long-term potentiation (LTP) in the NAc of adults compared with younger (P20-24) mice (Schramm *et al,* 2002). These data add to a growing body of literature on developmental regulation of synaptic plasticity. In this regard, the most studied region is the CA1 area of the hippocampus, where development of LTP induction by synaptic activity follows an inverted U-shaped curve. LTP induction peaks between postnatal day 15 and 30 (juvenile – early adolescence), and becomes more difficult to induce into

adulthood (Izumi and Zorumski, 1995). Differences in LTP induction in adolescent and adult mPFC remain to be explored. Wang and Gao elegantly examined the differences between GABA-ergic interneurons in juveline, adolescent and adult mPFC (Wang *et al,* 2009). Their principal finding is that fast-spiking interneurons undergo dramatic changes in glutamatergic receptors during adolescence, with a sharp decrease in the NMDAR/AMPAR ratio and the gradual loss of NMDARs (Wang *et al,* 2009). In monkeys it was shown that in the dorsolateral PFC the GABAA receptor-mediated mIPSPs from layer 2/3 continue to mature during adolescence (Hashimoto *et al,* 2009). Specifically, the decay time was shorter in adults, and there was a shift in the cumulative probability distribution.

In chapter 2, I showed using a synaptic proteomics study of the mPFC, that the largest group of developmentally regulated proteins is that of proteins associated with synaptic vesicle release, while there is no difference in the number of synaptic vesicles. I found a non-stoichiometric expression of various synaptic vesicle proteins that may indicate differences in release probability and hence short-term plasticity properties. These findings show that in the rat mPFC synaptic properties continue to mature during adolescence, but physiological research will be necessary to pinpoint the exact differences in synaptic properties between adolescent and adult mPFC.

Although in primates it is often described that overproduction and subsequent elimination of synapses is a hallmark of postnatal cortical development (Bourgeois *et al,* 1994; Huttenlocher, 1979; Rakic *et al,* 1986; Rakic *et al,* 1994), we find no evidence for this, as there are no developmental changes in levels of pre- or postsynaptic scaffolding proteins, and we do not find 'markers' for synapse elimination such as members of the complement system (Stevens *et al,* 2007). A weakness of our study is that we did not actually count synapses in the mPFC, but we estimated their presence on the basis of the levels of PSD-95 and Piccolo (a post- and presynaptic marker respectively) in total mPFC homogenates. Thus, I found no indication for synapse formation or elimination at the large scale. Synapse elimination may be a feature that is exclusive for the primate cortex, and may therefore not be found in rodent cortex. Another explanation is that in previous research spine counts were used to estimate synapse elimination, and since spines are postsynaptic protrusions, these may not represent functional synapses, and one may therefore overestimate the amount of synapse elimination. However, our results do point to a prominent role for presynaptic maturation of mPFC synapses during adolescent development, a process that might become affected by drug exposure.

3.2 Maturation of receptor levels

Another prominent feature of adolescent brain development is the maturation of receptor levels (Chapter 1). The adolescent maturation of most neurotransmitter receptors follows an inverted U-shape, with peak levels of receptors at the start of adolescence (around P35), and subsequent reduction to reach adult levels at the end of adolescence.

In Chapter 2 I measured glutamate receptors in the mPFC, and I found no evidence for differential expression of any of these. Perhaps we did not find developmental differences because those occur earlier in development.

In Chapter 4 I found that in the mPFC, α4β2* containing nAChRs decrease their expression during development. An unexpected result was that this decrease in expression does not stop at the end of adolescence, but continues until the last measured time point, which is P104. This developmental decrease of α4β2* was also suggested using α4 immunostaining in mice (Rogers *et al,* 1998). Specifically in the hippocampus, expression of α4 decreased from young adult (2-4 months of age) to very old mice (24-28 months of age) (Rogers *et al,* 1998). One of the ongoing developmental processes in the adolescent mPFC is activity-dependent elimination of synapses (Lichtman *et al,* 2000). In our proteomics dataset we do not find any evidence for the elimination of (mostly) glutamatergic synapses (see above), but that may still mean that the synapses or cells expressing nAChRs are eliminated, or that some other unknown developmental mechanisms are responsible for this age-dependent decline in receptor number.

3.3 IEG expression

Nowadays, the induction of IEGs such as c-fos and Egr1 (Zif268) is widely used as marker of neuronal activation to see which brain regions are activated in response to stimuli (Mattson *et al,* 2008; Schmidt *et al,* 2005). Immediate early gene (IEG) expression in the nucleus accumbens, the reward-center of the brain, following administration of cocaine has been described for the first time almost 20 years ago (Hope *et al,* 1992). Adolescents often show a different expression of IEGs in response to drug administration, but the magnitude and direction of these differences depend on the drug, brain region and immediate early gene studied (Andersen *et al,* 2001; Caster and Kuhn, 2009; Caster *et al,* 2005; Faria *et al,* 2008; Schochet *et al,* 2005). Most of the times, IEG expression seems exaggerated in adolescents compared with adults. Since IEG expression leads to an altered synaptic plasticity, for example increased Arc expression has

been shown to block the homeostatic scaling of AMPA receptors (Shepherd *et al,* 2006), the adolescent brain may be more vulnerable to drug-induced synaptic plasticity.

3.4 Connectivity between brain regions

During adolescence, connectivity between brain regions increases, and particularly the efferent projections from the mPFC to other brain regions continue to mature during adolescence (Witte *et al,* 2007). The glutamatergic output from the mPFC to the NAc, coming mostly from layer V of the mPFC, is modulated by dopamine from the VTA, and is generally believed to mediate drug seeking behavior (Kalivas *et al,* 2005). This connection is likely to be involved in cognitive functioning, such as in impulsivity and decision-making, since dopaminergic signaling in the PFC is important in increasing the signal-to-noise ratio by reducing the impact of irrelevant inputs to the PFC (Arnsten, 2006). During adolescence, not only the mPFC output to the NAc increases, but also the dopaminergic modulation of this connection undergoes developmental changes (Brenhouse *et al,* 2008). Compared with both juvenile and adult rats, adolescent rats show elevated dopamine D1 receptor expression on PFC-NAc projections (Brenhouse *et al,* 2008). Moreover, both D1 and D2 receptors on GABAergic interneurons (Tseng *et al,* 2007a; Tseng and O'Donnell, 2007b) and D1 receptor enhancement of NMDA function (Tseng and O'Donnell, 2005) in the PFC reach mature levels only during or shortly after adolescence, suggesting that mPFC output activity is indeed fine-tuned during late adolescence. Moreover, these maturational changes are believed to underlie the adolescent sensitivity to for example cocaine (Brenhouse *et al,* 2008). To date, however, it is not yet known whether adolescent exposure to drugs of abuse has long-lasting consequences for the mPFC-NAc connectivity, or its dopaminergic modulation.

In conclusion, the adolescent brain - and specifically the prefrontal cortex - continues to develop until young adulthood. The adolescent brain is more plastic, and drugs of abuse can exert larger and longer lasting effects compared to their effects on the adult brain. Considering the large amount of adolescents that start using drugs, it is of importance to study the long-term effects of adolescent drug use in more detail.

4. Functional consequences of adolescent drug exposure

As outlined in this chapter, how brain development is altered and more

specifically how adolescent drug exposure leads to long-term changes, is rather unexplored. There are many examples of differences in molecular, physiological or behavioral responses to drugs of abuse in adolescents compared with adults. Thus far, there are no clear examples of functional alterations in molecular or cellular mechanisms that lead to behavioral deficits brought about by adolescent drug exposure. Using a multidisciplinary approach, I investigated how adolescent nicotine exposure can lead to long-lasting cognitive deficits by studying the molecular and physiological properties of the mPFC, both one day and five weeks following adolescent nicotine exposure.

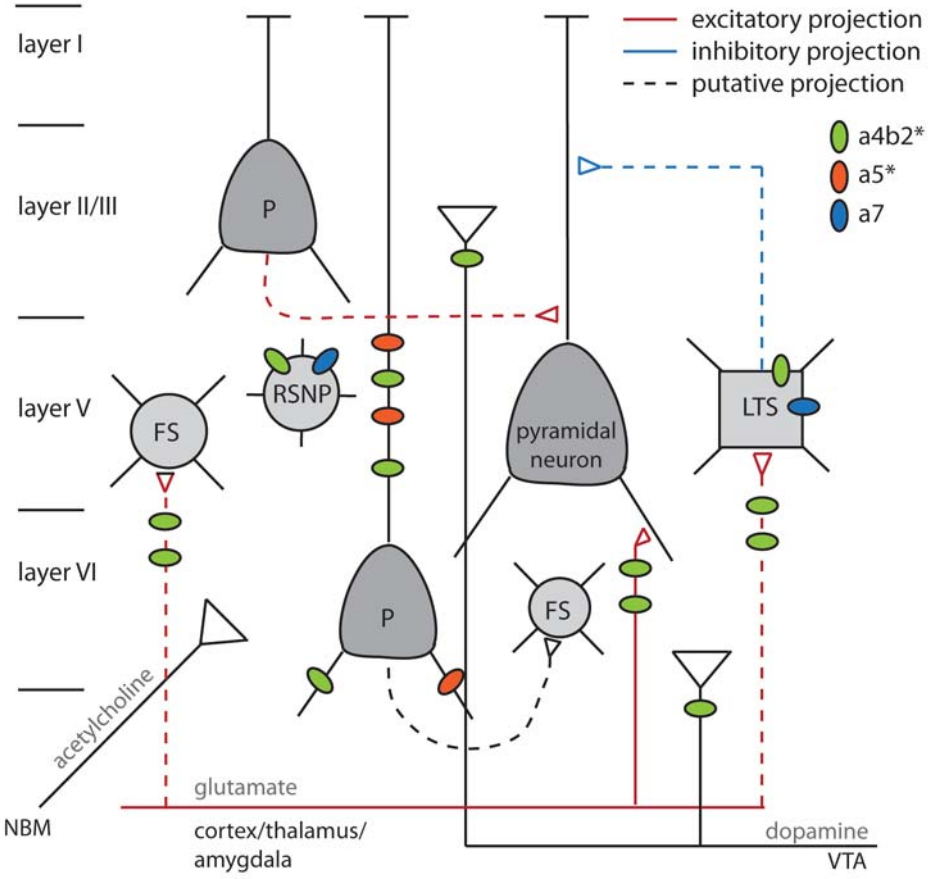


Figure 1. Schematic showing the localization of nAChRs in the mPFC. Modified from (Poorthuis et al, 2009). In the mPFC, nAChRs are only found on pyramidal neurons in layer VI, which also express α5* nicotinic receptors (Kassam et al, 2008). Both α4β2* and α7 receptors are expressed on non-fast spiking interneurons (Couey et al, 2007; Lambe et al, 2003), and input from the thalamus (Lambe et al, 2003) and the VTA (Marshall et al, 1997) can be modulated because of the presynaptic expression of nAChRs.

4.1 Short-term effects of adolescent drug exposure

4.1.1 Adolescent nicotine exposure affects protein expression

In Chapter 4 and 5, I found a number of proteins involved in glutamatergic transmission (mGluR2, GluN1, GluN2 and EAAT2), as well as cholinergic transmission (Chrna4, and Chrnb2) to be differentially regulated on the first day of abstinence after 10 days of nicotine injections. Although changes on both type of neurotransmitter systems were transient, as the direction of regulation was not present after 5 weeks of abstinence, only the proteins related to the glutamatergic system showed regulation in an opposite direction after 5 weeks of abstinence.

Short-term effects on the glutamatergic system

Because these proteomics changes in are relatively small (<10%), they could only partly be corroborated by immuno-blotting, but were shown to be specific for the adolescent treated group. This indicates that nicotine can differentially affect glutamatergic signaling in the mPFC. However, these effects seemed only partially long-lasting, since five weeks following adolescent nicotine exposure there are no differences in baseline EPSCs, or in AMPA/NMDA receptor ratio currents measured in layer V of the mPFC, despite the slight expression differences in NR1 and NR2B.

Changes in proteins related to glutamate signaling may be due to increased glutamate transmission caused by nicotine administration. Nicotine treatment has been shown to increase glutamate release in vivo (measured with microdialysis) as well as in synaptosomes via low-affinity α7 nicotinic receptors (Bancila *et al,* 2009; Konradsson-Geuken *et al,* 2009), but also via high-affinity α4β2 receptors (Lambe *et al,* 2003), each doing so via distinct mechanisms (for review (Mansvelder *et al,* 2009)).

Short-term effects on the nicotinergic system

In this respect, I found that adolescent animals also show an exaggerated upregulation of nAChRs in response to repeated nicotine injections when compared with adult animals (Chapter 4). This differential regulation of α4β2 nicotinic receptors is specific for the mPFC, since there is no difference in nAChR expression between adolescent and adult animals in other brain regions such as, for example, the primary visual cortex.

Nicotine acts through nicotinic receptors that are localized on various synaptic, but also largely on extrasynaptic sites in the mPFC (Figure 1) and nicotine administration is known to elicit dopamine release in the mPFC, when given systemically (Nisell *et al,* 1996) or locally (Marshall

et al, 1997). An increased number of nAChRs in the mPFC can therefore increase dopamine release in that area (Schultz, 2002). However, we observed that one day following adolescent nicotine exposure, the increased nAChR-expression did not translate into an increased nicotine-stimulated dopamine release. Thus far, we have yet to show that the upregulation of nAChRs leads to a functional difference in the mPFC. Perhaps the upregulation of nAChRs in the adolescent mPFC leads to other changes in the mPFC network, for example a different response to nicotine in terms of inhibitory input onto the pyramidal neurons, via activation of the nAChRs on GABAergic interneurons (Couey *et al,* 2007). However, it is difficult to state whether the changes we observed are caused by repeated nicotine administration or by withdrawal from nicotine.

4.1.2 Drug effects on synaptic plasticity during adolescence

Apart from the basal differences between the adolescent and adult brain, it is of interest to examine whether drugs of abuse are able to trigger differential changes in synaptic plasticity between adolescents and adults, as suggested by the differential IEG inductions upon drug exposure. For instance, Placzek et al. showed that dopamine neurons in the VTA were shown not to differ with respect to baseline physiological properties, but when current was injected, the adolescent VTA neurons would fire more action potentials than those from adult mice (Placzek *et al,* 2009). Also, DA neurons in the VTA from adolescents were more sensitive to nicotine-induced LTP (Placzek *et al,* 2009). Most other work addressing the acute effects of drugs on synapse function has been focused on the effects of alcohol in the hippocampus. Already 10 years ago, Pyapali et al. discovered that alcohol depresses the induction of LTP in the hippocampus of adolescent animals at doses that are ineffective in adults (Pyapali *et al,* 1999). Sabeti et al. added to this that in vivo alcohol exposure increases activity-dependent LTP at CA1 dendritic synapses (Sabeti and Gruol, 2008). LTP in the early adolescent hippocampus was increased compared with that of late-adolescent rats 24 hours following cessation of alcohol vapor. The reason for this increased LTP is likely to be the fact that alcohol induces less activation of interneurons in juvenile than in adolescent animals (Li *et al,* 2006). Specifically, the sensitivity of GABA(A) receptor-mediated inhibitory processes to alcohol increases with development. Alcohol can increase the firing of hippocampal interneurons in a dose-dependent manner, a process that is more pronounced in adults versus adolescents (Yan *et al,* 2009).

4.2 Long-term effects of adolescent nicotine exposure

4.2.1 Cognitive performance

In contrast to many reports showing an attention-enhancing effect of nicotine, in adolescents nicotine may not be that beneficial to cognitive functioning, since tobacco smoking in adolescents is associated with disturbances in working memory and attention (Jacobsen *et al,* 2005). Also, reduced attention-associated prefrontal cortical blood-oxygen level dependent (BOLD)-responses have been reported (Musso *et al,* 2007). These reports on the short-term effects of adolescent nicotine exposure in humans are complemented by our own work in rodents, in which we observed that adolescent nicotine exposure causes a long-lasting decrement in attentional performance, measured in the 5-choice serial reaction time task (5-CSRTT) (for review (Robbins, 2002)). The 5-CSRTT is designed to measure visuospatial attention, but also measures impulsive action, which is the inability to withhold from responding prematurely. Adolescent nicotine exposure has long-term consequences for impulsivity in the

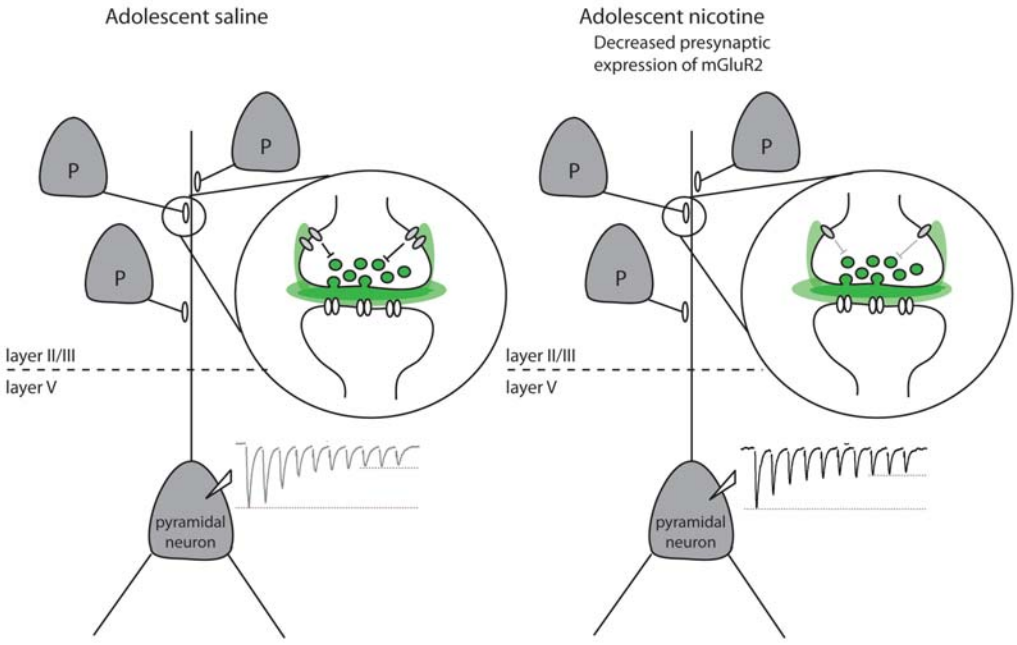


Figure 2. The adult mPFC network following adolescent saline/nicotine exposure. mGluR2 is a presynaptic autoreceptor that decreases glutamate release upon activation by spilled-over glutamate. Decreased expression of mGluR2 leads to a less reliable signal transduction, with decreased short-term depression under conditions of high-frequency stimulation, such as might occur during an attention task.

5-CSRTT, as adolescent nicotine exposed animals are almost twice as impulsive compared with their saline controls. I have investigated whether changes in synaptic protein expression exist that are responsible for these changes in behavior, and if so, whether they shed light on a mechanism of action.

4.2.2. Protein expression

Five weeks following adolescent, but not adult nicotine exposure I found that the metabotropic glutamate receptor (mGluR2) was downregulated in synaptic membranes of the mPFC, perhaps as an overcompensation following mGluR2 upregulation at one day of abstinence. mGluR2 is a presynaptic autoreceptor that can inhibit glutamate release from its own synapse, and thereby prevents other neurons from being hyper-excited (Pacheco Otalora *et al,* 2006). The downregulation of mGluR2 following adolescent nicotine exposure was functional, as it translated into a decreased mGluR2-dependent inhibition in EPSCs in the mPFC (Chapter 5, Figure 2). Given that mGluR2 activation mostly occurs following high-frequency stimulation, the downregulation of mGluR2 may lead to deficits in information processing, which is exemplified by a decreased short-term depression upon high-frequency stimulation in slices. It is therefore conceivable that adolescent nicotine exposure leads to deficits in filtering relevant signals in the mPFC and therefore leads to attentional deficits (Figure 2).

In addition to the changes in the glutamatergic system, we observed that five weeks following nicotine exposure, dopamine releasability was enhanced in the adolescent, but not adult mPFC (Chapter 3). Changes in dopamine signaling in the mPFC have been demonstrated previously to lead to cognitive or attentional deficits (Levy, 2008; Toda and Abi-Dargham, 2007). mGluR2 can modulate the release of glutamate, and the question arises whether the increased dopamine releasability in the mPFC observed five weeks following adolescent nicotine exposure can be attributed to the decreased mGluR2 expression at the synapse. It has been shown previously that the mGluR2/3 agonist LY379268 increases dopamine release in the mPFC when administered systemically, but not when administered locally (Cartmell *et al,* 2001). Similarly, we observed that the mGluR2/3 antagonist LY341495 has no effect on dopamine releasability in mPFC slices (unpublished observation). Therefore, it is unlikely that the decreased synaptic expression of mGluR2 in the mPFC has a direct effect on dopamine release. The possibility remains that also in other brain regions mGluR2 expression is altered, which subsequently causes increased dopamine releasability in the mPFC.

4.2.3 Stimulating mGluR2/3 in the mPFC rescues the attentional deficits following adolescent nicotine exposure

Recently, glutamate was found to play an important modulatory role in visuospatial attention and impulsivity, particularly in the mPFC (for review (Pattij *et al,* 2008a)). For example, in rats intracranial (intra-mPFC) blockade of NMDA receptors by 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) dose-dependently decreases attentional processing and increases impulsivity (Mirjana *et al,* 2004; Murphy *et al,* 2005). In humans, administration of an NMDA antagonist while performing an auditory attention task in an MRI scanner showed reduced activity in the prefrontal cortex and anterior cingulate cortex [35]. Thus, this strengthens the notion of PFC glutamate involvement in attentional processing. mGluRs have a subtle role in modulating glutamate transmission, which might explain why under normal conditions, administration of an mGluR2/3 antagonist had no significant effect on visuospatial attention (Semenova *et al,* 2007). In addition, the mGluR2/3 agonist LY379268 tended to improve visuospatial attention, but only in a strain of mice that exhibited poorer levels of visuospatial attention (Greco *et al,* 2005). The latter observation is in line with our current findings, that infusion of LY379268 into the mPFC only improves visuospatial attention in adolescent nicotine exposed animals that already exhibited poorer performance levels (Chapter 5). Although visuospatial attention and impulsivity are often correlated in the 5-CSRTT (Puumala *et al,* 1996), both lesion (Chudasama *et al,* 2003) and pharmacological (van Gaalen *et al,* 2006b) studies highlight that these cognitive functions can be functionally dissociated (for review (Robbins, 2002)). Further support comes from a study showing that rats selected on trait impulsivity did not differ in terms of visuospatial attention (Dalley *et al,* 2007a). Our data reveal a functional and causal relationship between mGluR2 downregulation in mPFC synapses following adolescent nicotine exposure and visuospatial attentional disturbances, whereas this relationship appears less clear-cut for impulsive behavior. Perhaps elevated mGluR2 levels following nicotine exposure at the end of the adolescent period compensate for nicotine's actions and inhibit neurotransmitter release (Pilc *et al,* 2008). Regardless, altered glutamatergic synaptic transmission in the mPFC plays a key role in attention and impulsivity (Mirjana *et al,* 2004; Murphy *et al,* 2005) and altered mGluR2 levels have been linked to several brain disorders, such as addiction and schizophrenia (Huang *et al,* 2007; Moreno *et al,* 2009; Xie *et al,* 2008). The sustained molecular and synaptic changes that result from nicotine exposure during adolescence and alter cognitive performance during adulthood give us

more insight in the etiology of attentional disorders.

5. Conclusion

In conclusion, adolescent nicotine exposure has deteriorating consequences, which seem to last a lifetime. The adolescent brain, and in particular the late-developing PFC is vulnerable to drug-induced alterations. We have shown that there are molecular differences between the mPFC of adolescent and adult rats, in which most of these developmental changes are related to synaptic vesicle proteins. Furthermore, adolescent nicotine exposure has short-term effects on the regulation of nicotinic receptors, and on a small subset of glutamatergic synaptic proteins. Our most prominent results are the long-term effects of adolescent nicotine exposure, in which we show that down-regulation of mGluR2 has functional consequences for synaptic functions and adult cognitive behavior.

In a broader perspective, this means that also for humans, adolescence may represent a period of increased vulnerability to develop drug addiction and cognitive impairment due to adolescent nicotine use. It is therefore important to educate adolescents about the potentially life-long consequences of starting to smoke when their brains are still developing. Meanwhile, future research should focus further on the molecular and neuronal network changes that occur following adolescent drug exposure and how these translate into long-lasting effects on behavior.